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**EUROPEAN PATENT APPLICATION**

21 Application number: 86104321.4

22 Date of filing: 27.03.86

51 Int. Cl.<sup>4</sup>: **C 07 K 15/00**  
**C 12 P 21/00, C 12 N 5/00**  
**C 12 N 15/00, G 01 N 33/577**  
**G 01 N 33/573**  
**/(C12P21/00, C12R1:91)**

30 Priority: 19.04.85 US 724991

43 Date of publication of application:  
29.10.86 Bulletin 86/44

84 Designated Contracting States:  
DE GB

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54 Monoclonal antibodies to human gastrointestinal cancer.

57 Diagnostic panels for human gastrointestinal abnormalities such as cancer using mouse monoclonal antibodies are disclosed. These panels can be used in diagnosis and in therapeutic applications such as colon cancer.

1 This invention was partially made with United States  
government support under CA 08748 awarded by the National  
Cancer Institute. The government has certain rights in this  
invention.

### 5 Background

10 This invention concerns monoclonal antibodies  
recognizing human gastrointestinal (GI) cells. The  
monoclonal antibodies recognize antigenic markers on normal  
as well as cancerous GI cells. Capable of distinguishing  
among normal GI cells as well as colon carcinomas, these  
mAbs are useful in diagnosis and prognosis of colon and  
15 gastrointestinal cancer. Examination of human GI specimens:  
tissues wastes, exudates, and fluids with these mAbs is a  
diagnostic procedure to probe for cancer of the  
gastrointestinal tract and especially colon cancer. These  
mAbs are of special importance because of the widespread  
20 occurrence of colon and stomach cancer.

In 1975 Köhler and Millstein introduced a  
procedure for the production of monoclonal antibodies (mAbs)  
using hybrid cells (hybridomas) which allows the production  
25 of almost unlimited quantities of antibodies of precise and  
reproducible specificity. Conventional antisera, produced

1 by immunizing animals with tumor cells or other antigens,  
contain a myriad of different antibodies differing in their  
specificity and properties, whereas hybridomas produce a  
single antibody with uniform characteristics. The  
5 Kohler-Millstein procedure entails the fusion of spleen  
cells from an immunized animal with an immortal myeloma cell  
line. From the fused cells (hybridomas), clones are  
selected that produce antibody of the desired specificity.  
Each clone continues to produce only that one antibody. As  
10 hybridoma cells can be cultured indefinitely (or stored  
frozen in liquid nitrogen), a constant supply of antibody is  
assured.

15 Antibodies are proteins that have the ability to  
combine with and recognize other molecules, known as  
antigens. Monoclonal antibodies are no different from other  
antibodies except that they are very uniform in their  
properties and recognize only one antigen or a portion of an  
antigen known as a determinant.

20 In the case of cells, the determinant recognized is an  
antigen on or in the cell which reacts with the antibody.  
It is through these cell antigens that a particular antibody  
recognizes, i.e. reacts with, a particular kind of cell.  
25 Thus the cell antigens are markers by which the cell is  
identified.

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These antigenic markers may be used to observe the normal process of cell differentiation and to locate abnormalities within a given cell system. The process of differentiation is accompanied by changes in the cell surface antigenic phenotype, and antigens that distinguish cells belonging to distinct differentiation lineages or distinguish cells at different phases in the same differentiation lineage may be observed if the correct antibody is available. Initial recognition of differentiation antigens came about through analysis of surface antigens of T-cell leukemias of the mouse and the description of the TL, Thy-1, and Lyt series of antigens. (Old, Lloyd J., Cancer Research, 41, 361-375, February 1981) The analysis of these T-cell differentiation antigens was greatly simplified by the availability of normal T cells and B cells of mouse and man and is relatively advanced. (See Patents #4,361,549-550; #4,364,932-37 and #4,363,799 concerning mAb to Human T-cell antigens). There is further experimentation to be done concerning differentiation antigens displayed on normal and neoplastic cells belonging to other lineages.

The preparation of hybrid cell lines can be successful or not depending on such experimental factors as nature of the inoculant, cell growth conditions, hybridization

1 conditions etc. Thus it is not always possible to predict  
successful hybridoma preparation with one cell line although  
success may have been achieved with another cell line.

5 Progress in defining surface antigens on melanocytes  
was made possible by the recently discovered technique of  
culturing melanocytes from normal skin (Eisinger, et al.,  
Proc. Nat'l. Acad. Sci. USA, 79 2018 (March 1982). This  
method provides a renewable source of proliferating cells  
10 for the analysis of melanocyte differentiation antigens.  
Likewise, a large number of cell lines derived from  
melanomas have now been established and these have  
facilitated the analysis of melanoma surface antigens. The  
advent of mAbs has greatly accelerated knowledge about the  
15 surface antigens of malignant melanoma. Cell markers on  
both melanomas and melanocytes have been identified. A  
panel of typing monoclonal antibodies has been selected  
which recognizes differentiation antigen characteristics at  
each stage of development in both melanocytes and melanomas.  
20 These differentiation antigens may be used to classify  
melanocytes and melanomas and to group them into  
characteristic sub-sets. Dippold et al. Proc. Nat'l. Acad.  
Sci. U.S.A. 77, 6114 (1980) and Houghton, et al. J. Exp.  
Med. 156, 1755 (1982). Immunoassay of melanocytes and  
25 melanoma cells within sub-sets is thus made possible.

Summary

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Cancers of the gastrointestinal tract are especially widespread; stomach cancer in Japan, colon cancer in the west and U.S.A. Early diagnosis would be desirable to prevent loss of life and prescribe alternatives to drastic surgery. Positive diagnosis can help to support the surgical decision. Cytohistological methods to date are not always successful. A panel group of mAbs of the present invention recognizing cancerous GI cells enables such a distinction. In addition, the panel distinguishes normal from cancerous cells.

15  
20  
25  
The invention thus comprises hybridoma cell lines producing mAbs recognizing human colon cancer cells, from the group of AS33, AS37, CLK314, CLH70, HT29/15, HT29/26, CLT307, CLT86, V-215, V-715. A preferred group comprises AS33, AS37, CLH70, CLK314, CLT86, CLT307, HT29-15, and HT29-26. These mAbs of the invention recognize colon or GI glycoprotein (gp) antigens molecular weights 25kd, 29kd and 95kd (mAbs CLH70, HT29/26 and CLT479 respectively). mAb CLT152 recognizes a protein antigen of 52 kd. The antigens for CLH6, CLT85, CLT174 and HT29/36 are heat stable and therefore probably glycolipids. CLT85, CLT479, CLT174, HT29/36, CLH68, CLT152 and HT29/15 are gamma sub one



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(gamma<sub>1</sub>) immunoglobulins. HT29/26 is a gamma sub 2A (gamma<sub>2A</sub>) immunoglobulin. HT29/36 is a gamma sub 3 (gamma<sub>3</sub>) immunoglobulin and CLT218, CLT307, CLT86 and CLH6 are mu immunoglobulins. (HT 29/36 is the same mAb as HT 29-36 or 29/36. HT 29/15 is the same mAb as HT 29-15 or 29/15 and HT 29/26 is the same mAb as HT 29-26 or 29/26. As 33 is the same monoclonal antibody as A-33 and AS37 is the same as A-37. In the tables below, CLH6 is the same mAb as 6, CLT86 is the same as 86, CLT85 is the same as 85, CLT307 is the same as 307, CLT479 is the same as 479, CLT174 is the same as 174, CLH 70 is the same as 70, CLT 15 is the same as 15, CLH70 is the same as 70, CLT152 is the same as 152. The following hybridoma cell lines and monoclonal antibodies produced therefrom namely: HT 29/15, HT 29/26, HT 29/36, CLH 6 (or 6), CLT 85 (or 85), CLT 479 (or 479), CLT 174 (or 174), CLH68 (or 68), CLT 152 (or 152), CLH 70 (or 70) CLT 218 (or 218), CLT 15 (or 15), CLT 307 (or 307) and CLT 86 (or 86) have been disclosed and claimed in a previously filed, co-pending application filed March 11, 1983, Serial No. 474,415 herein incorporated by reference. These are described in a publication Sakamoto et al \_\_\_\_\_ herein incorporated by reference.

#### Description

A preferred embodiment of the present invention is to test a human specimen as for example human body tissues, wastes, fluids and exudates with each of the monoclonal



antibodies of the panel. The cells are tested or contacted separately with each of the monoclonal antibodies in a series of dilutions. Thus, an assay for cancer is possible with minimal patient disruption. Indeed, the present invention permits testing of human GI waste specimens for cell fragments containing antigenic markers for the monoclonal antibodies. Entire cells need not be present. Cytohistological methods requiring whole cells are not always successful.

The monoclonal antibodies of the present invention were prepared by the Kohler-Millstein procedure wherein spleen cells from a mouse (or other mammal) immunized with human cancerous colon cells or pancreas from established human tumor cell lines of fresh tumor tissue were fused with mouse myeloma to form hybridomas. By serological screening, antibodies from these hybridomas were found which recognize differentiation antigens on normal bladder and/or cancerous bladder. Other tissues, both normal and cancerous, may be recognized as well by some of these monoclonal antibodies. A system of classification of normal as well as cancerous colon based on these differentiation antigens is now possible, and serological assays for tumors of the colon have been developed. These assays are of special use in the early diagnosis of gastrointestinal cancer especially colon cancer.

# TISSUE CULTURE:

Cultured human colon cancer cell lines came from Leibowitz and from the collection of J. Fogh at Sloan-Kettering Institute. Cultures of other established human cell lines and normal tissue cells have been described.

## PRODUCTION OF MOUSE MONOCLONAL ANTIBODIES

BALB/c mice were immunized with either a colon carcinoma or pancreas carcinoma cell line or with fresh colon cancer tissues. Subcutaneous and intraperitoneal injections of  $1 \times 10^6$  cells were given three to ten times at intervals of 2 weeks. Three days after the last injection, the fusion of immune spleen cells with mouse myeloma MOPC-21 NS/1 cells was performed as described. Culture supernatants were tested for antibody by the anti-mouse Ig mixed hemmagglutination assay (MHA) or Protein A assay (PA) on a panel of cultured cell lines of colon and other types of tissue cells. After subcloning five to six times, hybridoma cells were injected subcutaneously into nu/nu mice (Swiss background) and sera from mice with tumors were collected and used for serological, immunopathological and biochemical characterization. In general these methods have been described in Ueda et al. (1981) Proc. Natl. Acad. Sci. U.S.A. 78:5122, Dippold et al. (1980) Proc. Natl. Acad. Sci. U.S.A. 77:6114.

1 SEROLOGICAL PROCEDURES:

5 The MHA, on cultured cells using rabbit anti-mouse Ig and mouse anti-SRBC has been described. Absorption tests, assessment of heat stability and proteinase sensitivity and antibody subclass determination were also performed as described. See Dippold et al, Supra and Ueda et al. Supra, Pfreundschuh et al. (1978) Proc. Natl. Acad. Sci. USA 75:5122, Ueda et al. (1979) J. Exp. Med. 150:564

10 IMMUNOPATHOLOGICAL PROCEDURES

Immunofluorescent staining of cryostat sections with fluorescein conjugated goat anti-mouse Ig (Cappel Laboratories) was performed as described. (Fradet et al. (1984) Proc. Natl. Acad. Sci USA January \_\_\_\_\_.

15 Immunoperoxidase staining, using monoclonal antibody, peroxidase conjugated goat anti-mouse Ig and 3-amino-9-ethylcarbazol (AEC) (Histoset, Ortho Diagnostic system) was carried out following procedures recommended by the manufacturer.

20 IMMUNOPRECIPITATION PROCEDURES:

25 Antibodies were tested for immunoprecipitation activity by using detergent solubilized cell extracts labeled by [<sup>3</sup>H] glucosamine. Nonidet P-40 solubilization of cells and

immunoprecipitation procedures using *Staphylococcus aureus* have been described. Aliquots of  $2 \times 10^6$  [3H] cpm from unfractionated cell extracts were used. Precipitated molecules were extracted with 60  $\mu$ l of 0.01M Tris-HCl pH 7.2/2.0% NaDodSO<sub>4</sub>/ 12.0mg of dithiothreitol per ml/15% (weight/volume) sucrose/0.01% pyronin Y by heating 5 min at 100°C and were analysed by polyacrylamide gel electrophoresis. Dippold et al. Supra. Cairncross et al., (1962) Proc. Natl. Acad. Sci. USA 79:5641.

The assay of the present invention comprises contacting a tissue containing colon cells with the antibody recognizing colon cell antigens, preferably monoclonal antibodies to one or more cell antigens of the gastrointestinal antigenic system, and observing the immunoserological or immunopathological antigenic reaction between said monoclonal antibody and said antigen. In a preferred embodiment of the invention, the tissue sample to be contacted is gastrointestinal tissue and the antigenic reaction of the contacted tissue is observed by well known techniques such as immunofluorescence, Rosette formation with sheep or human red blood cells linked to Protein A or anti-Ig direct absorption and the like. In another embodiment of the present invention, the tissue to be assayed is first excised and is then either freshly, or

after being frozen or embedded in paraffin by methods well-known in the art, contacted with the monoclonal antibodies of the invention. Observation of the reaction is as before.

In another preferred embodiment of the present invention, the tissue to be assayed comprises the intact body of an individual or a whole portion thereof. The antibody, tagged with a radioactive or other energy-producing element, is administered to the individual, and the whole body or part thereof is scanned externally for localization of radioactivity at the site of cancerous gastrointestinal cells. In another preferred embodiment cancerous colon cells are located.

The present invention also makes possible the treatment of gastrointestinal tumors in a patient wherein the monoclonal antibody recognizing the cell antigen of cancerous colon or other cancerous GI cells, is administered to the patient in an amount effective to inhibit the growth or proliferation of cancer cells. In a preferred embodiment of this method, the antibody is tagged with a potentially tissue destructive agent which causes destruction of the cancer cells. Examples of tissue destructive agents comprise chemotoxic agents, chemotherapeutic agents

1 including vaccines, radionuclides, toxins, complement  
activators, clotting activators and the like. These  
examples are for illustrative purposes only and are not  
meant to limit the scope of the invention.

5 The following examples are intended to illustrate the  
invention without limiting same in any manner especially  
with respect to substantially functional equivalents of  
hybridomas, monoclonal antibodies and cell lines described  
10 and claimed herein.

The monoclonal antibodies selected for use in the  
present invention were derived from spleen cells of mice  
immunized with whole cells of colon carcinoma cell lines  
15 such as Tallevi and HT-29 fresh tumor cell lines or  
pancreatic tumor cell lines by fusion methods well known in  
the art.

20 A group of monoclonal antibodies which were found to  
recognize specific cell antigens of gastrointestinal cells,  
was selected as the gastrointestinal panel. This panel and  
other mAbs and the antigenic systems recognized are given in  
Tables I, II, III and IV. Heterogeneity of human colon  
carcinoma is therein noted. The table data point out and  
25 define the heterogeneity of colon carcinomas.

1 Gastrointestinal antigenic systems are defined by these  
mAbs as determined by serological analysis with over 70  
tumor cell lines; 18 colon cancers, over 50  
non-gastrointestinal cancers as well as immunopathology on  
5 frozen sections of normal adult and normal fetal tissue and  
cancer tissue. (See Table I, II, III and IV Reactivity with  
tissue of cancer patients is shown in Table V)

10 Eight monoclonal antibodies to cell surface antigens of  
human colon carcinoma were obtained by immunization with  
cultured human colon and pancreas carcinomas or with lysates  
of colon cancer cells. The distribution of the antigens  
detected was analysed on 164 normal and malignant cell lines  
(Table III) and on frozen sections of normal adult and fetal  
15 tissues (Table IV). Fifty five colon carcinomas and normal  
colonic tissue from the same patient were also examined  
(Table V).

20 One very restricted antigen, V-215 (gp140), were  
detected only on colon and four other cancer cell lines  
(Table III). In several patients, the antigen were  
expressed only on colon cancers but not in normal adjacent  
colon tissues (Table V).



1 A-33 antigen was found only on colonic and pancreatic  
cancer cell lines, and not in any normal adult tissues, it  
was present on both tumor and normal adjacent colonic  
tissues (Tables III, IV and V).

5 K-314 antigen (gp170) was only on colon and a few lung  
cancer cell lines (Tables III). In immunopathology, the  
antigen was not found in any normal adult tissues except  
some part of the proximal tubules of the kidney (Table IV).

10 A-37 antigen was on colon, some renal and hematopoietic  
cell lines but was found only in the proximal tubules of  
kidney in immunopathology staining (Tables III and IV).

15 HT-29-15 antigen (H-15) (Tables I-IV) was detected on  
colon, breast and lung cancer cell lines and also, in  
certain patients, was expressed on colon cancer tissues but  
not on their normal counterparts (Table V). H-15  
determinants are carried on a high molecular weight  
glycoprotein and are neuraminidase-sensitive.

20 V-715 antigen (gp120) was expressed on colon, lung and  
renal cancers (Tables III and IV). V-715 antigen has a  
similar serological, and immunopathological characteristics  
25 with the Adenosine deaminase protein. H-70 (Table I-IV)  
antigen (gp29) was expressed on colon, lung, renal cancer  
and neuroblastoma cell lines.

1 HT-29-26 antigen (gp31) (H-26) was detected on almost  
all the epithelial tissues, but not in other tissues (Tables  
I-IV).

5 None of the antigens were related to A, B, H, I or  
Lewis blood group specificities.

#### Example I

10 Several of the antigens, as defined by the monoclonal  
antibodies of the panel, are expressed differentially by  
cell populations within the adult GI system. CLT152 antigen  
is expressed by epithelial cells of the GI mucosa of  
esophagus, stomach, small intestine and colon, but is not  
found in other adult tissues. CLH70, CLT307, CLT86 and  
15 CLH68 antigens are expressed by adult stomach, small  
intestine and colon. CLT218 is expressed by adult small  
intestine and colon. HT29/26 is expressed by colon and some  
cells of small intestine in the adult. CLT15 also is  
expressed by normal colon epithelium as well as some upper  
20 GI cells except stomach in adult tissues. Thus the mAbs  
antigens HT29/26, CLT15, CLT218, CLH70, CLT307, CLT86 and  
CLH68 occur in adult colon epithelial cells; they vary among  
themselves in their pattern of distribution within the rest  
of the GI tract. There is some limited expression of these  
25 antigens in epithelial cells of other tissues as well [See

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1 Table II]. Thus, for example, CLT218, CLT86, HT29/26  
antigens are expressed on bronchial epithelium whereas CLH6,  
HT29/36 and HT29/15 are not. Thus, the panel antibodies  
5 differ in their expression on normal cells even as to  
similar cells of other tissues.

10 It is important that the mAbs CLH6, CLT85, and 29/36 do  
not react with normal adult tissue in immunopathology of  
frozen tissue sections but do react with distinct subsets of  
colon adenocarcinomas.

15 Serologically CLT85 reacts with approximately 11 of 17  
colon cancer lines, and CLH6 with approximately 8 out of 17  
colon cancer cell lines. CLT85 and CLH6 show no reaction  
with normal adult cells in serology.

20 From 23 further Köhler-Milstein fusions done as above  
of NS/1 myeloma with spleen cells, 8 more antibody producing  
clones were selected for further detailed analysis as  
discussed below. The serological specificities of these  
antibodies were tested on a panel of 154 established human  
cancer cell lines and on short term cultures of 10 human  
fibroblast and kidney epithelial cells (Tables III and IV).  
25 The immunopathological specificities of these antibodies  
were determined on a panel of human adult and fetal tissues,  
as well as normal colon epithelium and colon cancer tissues  
from 55 patients (Table V).

1 Monoclonal antibodies V-215, K-314 and V-715 were  
obtained after immunization with fresh colon specimen;  
antibodies A-33, A-37 were obtained after immunization with  
pancreas cancer cell lines AsPc-1, and antibodies H-15, (HT  
5 29/15 or HT29-15), H-70 and H-26 (HT 29/26 or HT29-26) were  
obtained after immunization with colon cancer cell line  
HT-29.

10 The heavy chain subclass of the eight antibodies are:  
V-215, gamma-1; A-33, gamma-2b; K-314, gamma-1; A-37,  
gamma-1; H-15, gamma-1; H-70, mu; H-26, gamma-2a.

#### Example II

15 V-215: Antibody V-215 react with 9/17 colon cancer cell  
lines with a strongest titer of  $5 \times 10^4$  against SW-1417 colon  
cancer cell line by rosetting. One lung, one ovarian, one  
terato-carcinoma and one melanoma cell lines were positive  
but all 152 other cell ines tested were negative in direct  
and absorption tests (Table III). The antigen was detected  
20 on secretion of bronchial epithelium and uterine  
endometrium, but not any other adult or fetal tissues tested  
(Table IV). In immunoperoxidase staining of the frozen  
section from 55 patients, V-215 was negative with normal but  
positive with colon tumor in 7 patients (Table V).

1 V-215 antigen was immunoprecipitated from [<sup>3</sup>H]  
glucosamine labelled cell extracts from colon cancer cell  
line SW-1417. The molecular weight is 140000 as estimated  
by polyacrylamide gel electrophoresis.

5  
Example III

A-33: Antibody A-33 is an IgG2b antibody that reacts with  
5/17 colon carcinomas and 1/3 pancreatic carcinoma (AsPc-1)  
10 with a titer of 10. A-33 also reacts with 3/6 T cell  
leukemia cell lines; all 155 other cell types tested were  
negative. Correlation between A-33 and T cell related  
antigens; OKT-6, T37,1, OKT-4, T,11, CL3-3 (13), CL3-40  
(13), was examined by the inhibition tests and by rosetting  
15 assay on immunizing cell line AsPc-1 and all the antigens  
were negative in both tests.

Antibody A-33 react with normal adjacent colonic mucosa  
and carcinoma of the colon cancer patient (Table V). One  
20 out of 5 pancreatic mucosa and pancreas cancer of one  
patient was also positive with the antigen. A-33 did not  
react with any other tissue sections examined on  
immunopathology staining (Table IV).

1 The antigen was not destroyed by heating at 100°C for 5  
minutes and it was present in the chloroform/methanol  
extract of AsPc-1 cells. In immunoprecipitation experiments  
using cell extracts labelled with [<sup>3</sup>H]glucosamine,  
5 radioactivity was precipitated that migrated at the dye  
front in 9% acrylamide gels. These properties strongly  
suggest that the antigen is a lipid.

#### 10 EXAMPLE IV

K-314: Antibody K-314 reacted with 13/17 colon carcinomas  
and 3/3 pancreas carcinomas, 7/25 lung carcinomas, 1/10  
bladder carcinomas, and 3/5 chorio and teratocarcinomas; the  
other 137 cell lines tested were negative (Table III).

15 In tissue sections, antibody K-314 reacted with lung,  
uterus and heterogeneous population of gastrointestinal  
tract epithelial cells of normal adult and fetal tissues  
(Table IV). Among the gastrointestinal cancer patients,  
20 K-314 is present in carcinoma but not in normal adjacent  
mucosa in 11 colon cancer, 5 metastatic colon cancer to the  
liver and 3 pancreas cancer patients (Table V).

25 K-314 was immunoprecipitated from [<sup>3</sup>H]glucosamine  
labelled cell lysate of AsPc-1. The molecular weight of the  
antigen is 170000 as estimated by polyacrylamide gel

1 electrophoresis.

EXAMPLE V

5 H-15: Antibody H-15 (HT-29-15) is an IgG1 antibody that  
reacts with 12/17 colon cancers, 2/3 pancreatic cancers, 2/2  
Hepatic and biliary cancers, 4/5 lung cancers, 1/8 bladder  
cancers, 2/4 ovarian cancers and weak rosetting with one  
melanoma and one renal cancer cell lines (Table III). H-15  
is found in lung and in some proportion of gastrointestinal  
10 mucosa in tissue sections (Table IV). H-15 is positive in  
cancer but negative in normal counterpart of the same  
patient in 8 colon cancers, 3 metastatic colon cancers and  
in 2 pancreas cancers (Table V).

15 The antigen was not destroyed by heating at 100°C for 5  
minutes and was proteinase resistant. The antigen  
disappeared after treatment with neuraminidase. In  
immunoprecipitation with [<sup>3</sup>H] glucosamine, weak broad band  
of molecular weight over 200000 is observed.

20 Example VI

A-37: A-37 is present in 5/17 colon cancers and 3/3  
pancreatic cancers, 3/20 renal cancers, 3/5 chorio- and  
25 teratocarcinomas and in 15/25 hematopoietic cells tumors



1 (Table III). In tissue sections, the antigen was found only  
on proximal tubules of the kidney but not in any other  
tissues tested (Table IV). This antigen is also heat  
stable, proteinase and neuraminidase resistant.

5

#### Example VII

V-715: V-715 antigen is in 8/17 colon cancers, 5/25 lung  
cancers, 1/10 bladder cancers and in almost all renal cancer  
10 cell lines (Table III). In tissue sections, V-175 is  
present in proximal tubules of the kidney, but not in normal  
gastrointestinal tract cells (Table IV). The antigen is  
found on 9 colon and pancreas cancer specimen and on 4  
normal adjacent colon and pancreas mucosa of those cancer  
15 patients (Table V). The antigen is a glycoprotein and the  
molecular weight is 120000.

The serology pattern with the cell lines and the  
immunopathology staining pattern with the tissues are very  
20 similar to the Adenosine deaminase binding protein which was  
detected by renal cancer monoclonals (Andy, Robin J., et al.  
(1984) J. Biol. Chem. 259:12844). Since V-175 is not  
detected in normal colon, the determinant of V-715 is likely  
to be the same as the epitope detected by monoclonal S-23.

25

Example VIII

1 H-70: H-70 is in 13/17 colon cancer cell lines and in  
several other epithelial cancer cell lines. H-70 is also on  
3/5 neuroblastoma cell lines. H-70 is detected in  
5 epithelial tissues in immunopathology. Immunoprecipitation  
with [H]glucosamine was performed to determine its molecular  
weight as 31000.

Example IX

10 H-26: Antigen H-26 (HT-29-26, C-26) is in almost all  
epithelial cancer cell lines but is not present in any  
neuroblastoma, melanoma or astrocytoma cell lines (Table  
III).

15 In immunopathology H-26 is present in all epithelial  
cells and in kidney, it is present on distal and collecting  
tubules (Table IV). H-26 is a glycoprotein and its  
molecular weight is 29000.

20 Thus normal versus neoplastic cells of the colon, GI,  
and pancreas can be differentiated and assayed by contacting  
a specimen from a human patient with each of the monoclonal  
antibodies of the panel in serial dilution, and observing  
any antigen antibody reaction by any of the methods cited.  
25 Although specific hybridomas producing monoclonal antibody

1 against gastrointestinal cell antigens are presented, it is  
obvious that the present invention encompasses all the mAbs  
exhibiting the characteristics described therein, especially  
the embodiment describing reaction with normal as well as  
5 tumor cell antigens of the GI tract.

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One of the preferred panels of the invention for colon cancer is:

SUMMARY OF MOUSE MONOCLONAL ANTIBODIES - COLON PANEL

Monoclonal Antibody (Ig subclass)	ATCC#	Molecular	Additional notes
AS33 (IgG2b)	HB 8779		N Colon/some Colon Ca.
AS37 (IgG1)	HB 8778		
CLH70 (IgG2b)	HB 8245	Mr29,000	
CLK314 (IgG1)	HB 8780		
CLT86 (mu)	HB 8252		
CLT307 (mu)	HB 8251		
HT29-15 (IgG1)	HB 8246	Mr 200,000	
HT29-26 (IgG2a)	HB 8247	Mr40,000	epithelial cell marker

Changes in cell antigens are associated with different stages of differentiation and different stages of cancer. Thus this invention technique defined cell antigens associated with differentiation and cancer of the GI tract and the pancreas.

Legend to Table I

Serological reaction of colon panel monoclonal antibodies with human tumor cell lines of various tissues by rosette formation with human red blood cells conjugated with

1 rabbit anti-Ig, Dippold Supra

where 0 = no reaction by rosette formation or  
absorption

5 2 = positive rosette reaction at less than  
1,000 fold dilution antibody  
supernatant

3 = positive rosette reaction at greater  
than 1,000 fold dilution antibody  
10 supernatant

1 = positive reaction by absorption test  
only.

15 If there is no rosette reaction, the absorption test was  
done. Thus if a mAb was negative for the rosette reaction  
but absorbed onto the test antigen system it was deemed to  
be a positive reaction such that

20 1 = positive reaction by the absorption  
test though mAb gives a negative test  
for rosette formation

25 i.e. 0 test for rosette reaction is further tested by the  
absorption test. Therefore 0 on this table indicates no  
reaction by either absorption or rosette reactions. For

1 comparison, mAb 19.9 was obtained from H. Kaprowski and  
assayed as well alongside the mAbs of the present invention  
Atkinson, B.F. et al., Cancer Research, 42:4820-4823(1982).

5 In Table I actual titers are included.

Immunogen for CLT series is Tallevi, for HT and CLH  
antibodies the immunogen is HT-29.

10 Legend to Table II

Immunopathological reaction of some of the colon panel  
monoclonal antibodies with fetal and adult normal human  
tissue and cancer tissue in frozen section by indirect  
15 immunofluorescence.

0 = no reaction

⊕ = positive reaction

⊙ = heterogeneous reaction within the tissue

20 The following monoclonal antibody-producing-hybridoma  
cell lines are maintained on deposit at Sloan-Kettering  
Institute for Cancer Research, 1275 York Avenue, New York,  
New York 10021 namely:

25 V-215, K-314, V-715, As-33, As-37, CLH 70, CLK  
314, CLT 86, CLT 307, HT 29-15 and HT 29-26.

Serological Reaction of Monoclonal Antibodies  
Produced from Human Colon Tumor Immunogen With Various  
Human Cancer Cell Lines

[illegible]



Serology

Serological Reaction of Monoclonal Antibodies  
Produced from Human Colon Tumor Immunogen With Various  
Human Cancer Cell Lines

IMMUNIZING TUMOR: COLON

	CLH	CLT	CLT	CLT	CLH	CLT	CLH	HT29	HT29	HT29	CLT	CLT	CLT	CLT	
CELLS TESTED	6	85	479	174	68	152	70	-15	-26	-36	218	15	307	86	19.9
Breast Ca.:															
MDA MB 361	0	0	0	0	0	0	3	3	0	0	0	0	0	0	0
MDA MB 231	0	0	0	0	0	0	2	3	3	0	0	0	3	0	0
BT 20	0	0	0	0	0	0	0	3	3	0	0	0	0	0	0
CAMA	0	0	1	1	0	0	0	2	0		3	3	3	3	0
SK-BR-3	0	0	0	0	0	0	0	3	0		0	0	0	0	0
ALAB	0	0	0	0	0	0	3	3			0	0	0	0	0
MCF-7	0	0	0	0	0	0	0	3	3	3	3	0	0	3	0
Kidney Ca.:															
SK-RC-6	0	0	0	0	0	0	0	0	3	0	0	0	0	0	
-7	0	0	0	0	0	0	0	0	3	0					0
-29	0	0	0	0	0	3	0	0	3	0	0	0	0		0
-4	0	0	0	0	0	0	0	0	3	0	0	0	0		
Ovary Ca.:															
SK-OV-3	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
ROAC	0	0	0	0	0	0	3	0	3		0	0	0	0	0
2774	0	0	0	0	0	0	0	0	3		0	0	0	0	0
SW 626	0	0	3	3	0	0	0	3	3		3	0	3	3	0
Snustak	0	0	0	0	0	3	0	3	3		3	3	3	3	0
Turanek	0		2	0	0	3	2	3	3	0	0	0	0	3	10
Uterine Ca.:															
ME180	0	0	0	0	0	0	0	0	3	3	0	0	0	0	0
Chorioepithelium:															
SVOC	0	0	2		0	0	0	0	3		0	0	3	0	0

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Table I  
Serology

Serological Reaction of Monoclonal Antibodies  
Produced from Human Colon Tumor Immunogen With Various  
Human Cancer Cell Lines

IMMUNIZING TUMOR: COLON

CELLS TESTED	CLH	CLT	CLT	CLT	CLH	CLT	CLH	HT29	HT29	HT29	CLT	CLT	CLT	CLT	
	6	85	479	174	68	152	70	-15	-26	-36	218	15	307	86	19.9
Lung Ca:															
SK-IC-3	0	0	2	0	0	0	3	3	3	0	0	0	0	0	0
-4	0	0	0	0	0	0	0	3	3	2	0	0	0	0	10
-5	1	0	0	0	0	0	0	3	3	0	0	0	0	0	0
-6	0	0	0	0	0	0	3	2	0	0	0	0	2	0	0
-7	0	0	0	0	0	0	0	0	3	2	0	0	0	0	0
-8	0	0	0	2	0	0	0	0	3	3	0	0	0	0	0
-13	0	0	0	0	0	0	2	0	3	2	0	0	0	0	0
Lawson	0	1	1	1	0	3	2	3	3	2	3	0	0	3	10 <sup>3</sup>
Bladder Ca:															
T-24	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0
TOC SUP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
253J	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0
639V	0	0	0	0	0	0	0	0	3	3	0	0	0	0	0
486P	0	0	0	0	0	0	3	3	3	0	0	0	0	0	0

## Table II

## Immunopathology

### Normal Tissue Distribution of the Monoclonal Antibodies Produced from Human Colon Tumor Immunogen

[illegible]

TABLE II

Immunopathology

Normal Tissue Distribution of the Monoclonal  
Antibodies Produced from Human  
Colon Tumor Immunogen

## A. FETAL TISSUES (Cont'd.)

	CLT 85	CLH 28	HT 6	HT 29/36	HT 29/15	CLT 479	CLT 15	CLT 174	CLT 86	CLH 70	CLT 152	19.9
OVARY	0	0	0	0	0	0	0	0	0	0	0	0
Germ. Cells	0	0	0	0	0	0	0	0	0	0	0	0
Connect. T.	0	0	0	0	0	0	0	0	0	0	0	0
FALLOP. T.	0	0	0	0	0	0	0	0	0	0	0	0
UTERUS	0	0	0	0	0	0	0	0	+	+	0	0
Endometrium	0	0	0	0	0	0	0	0	+	+	0	0
Myometrium	0	0	0	0	0	0	0	0	0	0	0	0
CERVIX	0	0	0	0	0	0	0	0	+	0	0	0
Endocervix	0	0	0	0	0	0	0	0	+	0	0	0
Exocervix	0	0	0	0	0	0	0	0	±	0	0	0
SKIN	0	0	0	0	0	0	±	0	+	0	+	0
Epidermis	0	0	0	0	0	0	±	0	±	0	±	0
Melanocytes	0	0	0	0	0	0	0	0	0	0	0	0
Breast Gland	0	0	0	0	0	0	0	0	0	0	0	0
Sebac. Gld.	0	0	0	0	0	0	0	0	0	0	0	0
Hair Fol.	0	0	0	0	0	0	0	0	0	0	0	0
Dermis C.T.	0	0	0	0	0	0	0	0	0	0	0	0
BRAIN	0	0	0	0	0	0	0	0	0	0	0	0
Neurons	0	0	0	0	0	0	0	0	0	0	0	0
Glial Cells	0	0	0	0	0	0	0	0	0	0	0	0
Dendrites	0	0	0	0	0	0	0	0	0	0	0	0
LYMPH NODE	0	0	0	0	0	0	0	0	0	0	0	0
BLOOD VES.	0	0	0	0	0	0	0	0	0	0	0	0
Endoth. Cel.	0	0	0	0	0	0	0	0	0	0	0	0
Smooth Ms.	0	0	0	0	0	0	0	0	0	0	0	0
SOFT TIS.	0	0	0	0	0	0	0	0	0	0	0	0
SECRETION	±	0	0	0	0	+	0	+	+	+	+	+

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TABLE II

SK 340  
4/12/85Immunopathology

Normal Tissue Distribution of the Monoclonal  
Antibodies Produced from Human  
Colon Tumor Immunogen

A. FETAL TISSUES (CONT'D.)

	CLT 307	CLT 218	HT 29/26	CLH 66
LUNG	+	+	+	0
Bronchial				
Epithelium	+	+	+	0
Cartilage	0	0	0	0
Pneumocytes	0	0	0	0
Connect. Tis	0	0	0	0
HEART	0	0	0	0
THYMUS	0	0	0	+
Hassall's C.	0	0	0	+
Thymocytes	0	0	0	0
SPLEEN	0	0	0	0
White Pulp	0	0	0	0
Red Pulp	0	0	0	0
LIVER	+	+	+	+
Hepatocytes	0	0	0	0
Biliary Epi				
Cells	+	+	+	+
GALLBLAD.	+	+	+	+
ESOPHAGUS	+	±	±	±
STOMACH	±	+	0	+
SMALL INT.	0	0	±	+
COLON	+	+	+	+
PANCREAS	+	+	+	0
Exocrine	+	+	+	0
Endocrine	0	0	0	0
KIDNEY	0	+	+	0
Glomerulus	0	0	0	0
Prox. Tub.	0	0	0	0
Distal Tub.	0	+	+	0
Collec. Tub	0	+	+	0
URETER	+	+	+	+
UR. BLAD.	+	+	+	+
ADRENAL	0	0	0	0
Cortex	0	0	0	0
Medulla	0	0	0	0
TESTES	0	0	0	0
Germ. Cells	0	0	0	0
Endoc. Cel.	0	0	0	0
Connect. T.	0	0	0	0



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TABLE IIA  
SUMMARY OF TISSUE DISTRIBUTION AND REACTIVITY OF MONOCLONAL ANTIBODIES AGAINST  
COLON CANCER BY IMMUNOPATHOLOGY IN NORMAL FETAL, NORMAL ADULT AND CANCER TISSUE IN HUMAN SPECIMENS

SK 340

4/12/85

## MONOCLONAL ANTIBODIES

NORMAL FETAL TISSUES	85	28	6	29/36	29/15	479	15	174	86	70	152	307	218	29/26	68
KIDNEY															
Glomerulus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Proximal Tubules	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Distal Tubules	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Collecting Tubules	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
URETER	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
URINARY BLADDER	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ADRENAL															
Cortex	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Medulla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TESTES															
Germ Cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Endocrine Cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Connective Tissue	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OVARY															
Germ Cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Connective Tissue	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FALLOPIAN TUBES	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UTERUS															
Endometrium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myometrium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CERVIX															
Endocervix	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Exocervix	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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TABLE IIA  
SUMMARY OF TISSUE DISTRIBUTION AND REACTIVITY OF MONOCLONAL ANTIBODIES AGAINST  
COLON CANCER BY IMMUNOPATHOLOGIC IN NORMAL FETAL, NORMAL ADULT AND CANCER TISSUE IN HUMAN SPECIMENS  
MONOCLONAL ANTIBODIES

SK 840  
4/12/85

NORMAL FETAL TISSUES														
	85	23	5	29/36	29/15	479	15	174	86	70	152	307	218	29/26
LUNG	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bronchial Epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cartilage	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pneumocytes	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Connective Tissue	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HEART	0	0	0	0	0	0	0	0	0	0	0	0	0	0
THYROID	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hassall's Corpuscles	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thymocytes	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SPLEEN	0	0	0	0	0	0	0	0	0	0	0	0	0	0
White pulp	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Red pulp	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LIVER	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hepatocytes	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Biliary Epith. Cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GALLBLADDER	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ESOPHAGUS	0	0	0	0	0	0	0	0	0	0	0	0	0	0
STOMACH	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SMALL INTESTINE	0	0	0	0	0	0	0	0	0	0	0	0	0	0
COLON	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PANCREAS	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Exocrine:	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Endocrine	0	0	0	0	0	0	0	0	0	0	0	0	0	0



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SUMMARY OF TISSUE DISTRIBUTION AND REACTIVITY OF MONOCLONAL ANTIBODIES AGAINST  
COLON CANCER BY IMMUNOPATHOLOGY IN NORMAL FETAL, NORMAL ADULT AND CANCER TISSUE IN HUMAN SPECIMENS

4/12/85

TABLE IIA

## MONOCLONAL ANTIBODIES

NORMAL FETAL TISSUES	85	28	6	29/36	29/15	479	15	174	86	70	152	307	218	29/26	68
SKIN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Epidermis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Melanocytes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sweat Gland	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sebaceous Gland	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hair follicle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dermis Connective Tissue	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BRAIN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Neurons	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cilial Cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dendrites	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LYMPH NODE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BLOOD VESSEL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Endothelial Cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Smooth Muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SOFT TISSUE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SECRETION	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE IIA

SUMMARY OF TISSUE DISTRIBUTION AND REACTIVITY OF MONOCLONAL ANTIBODIES AGAINST COLON CANCER BY IMMUNOPATHOLOGY IN NORMAL, FETAL, NORMAL ADULT AND CANCER TISSUE IN HUMAN SPECIMENS

SK 340  
4/12/85

MONOCLONAL ANTIBODIES

	85	28	6	29/36	29/15	479	15	174	86	70	152	19.9	307	218	29/26	68
<b>NORMAL ADULT TISSUE</b>																
<b>KIDNEY</b>																
Glomerulus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Proximal Tubules	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Henle's Loop	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Distal Tubules	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Collecting Tubules	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>URETER</b>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>URINARY BLADDER</b>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>ADRENAL</b>																
Cortex	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Medulla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>THYROID</b>																
Epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Colloid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>BREAST</b>																
Duct Cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acinar Cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Connective Tissue	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>PROSTATE</b>																
Epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stroma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>TESTES</b>																
Germ Cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Endocrine Cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Connective Tissue	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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Table II A

SK 340  
4/12/85

SUMMARY OF TISSUE DISTRIBUTION AND REACTIVITY OF MONOCLONAL ANTIBODIES AGAINST COLON CANCER BY IMMUNOPATHOLOGY IN NORMAL FETAL, NORMAL ADULT AND CANCER TISSUE IN HUMAN SPECIMENS

MONOCLONAL ANTIBODIES

Tissue	Normal Adult Tissues														
	85	28	6	29/36	29/15	479	15	174	86	70	152	19.9	307	218	29/26
LUNG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bronchial Epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cartilage	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glandular Epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pneumocytes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Connective Tissue	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HEART MUSCLE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SPLEEN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
White pulp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Red pulp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LIVER	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hepatocytes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Biliary Epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sinusoids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GALLBLADDER	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ESOPHAGUS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
STOMACH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SMALL INTESTINE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
COLON	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G.I. smooth muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PANCREAS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Exocrine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Endocrine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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Table 11A

SK 340  
4/12/85

SUMMARY OF TISSUE DISTRIBUTION AND REACTIVITY OF MONOCLONAL ANTIBODIES AGAINST  
COLON CANCER BY IMMUNOPATHOLOGY IN NORMAL FETAL, NORMAL ADULT AND CANCER TISSUE IN HUMAN SPECIMENS

	MONOCL ADULT TISSUES	85	28	6	29/36	29/15	479	15	174	86	70	152	19.9	307	218	29/26	68
OVARY	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Uterine Cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Connective Tissue	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FALLOPIAN TUBES	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UTERUS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Endometrium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myometrium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CERVIX	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Endocervix	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Exocervix	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PLACENTA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cytotrophoblasts	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Syncytiotrophoblasts	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sinusoids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SKIN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Epidermis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Melanocytes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sweat Gland	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sebaceous Gland	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dermis Connective Tis.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BRAIN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Neurons	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glial Cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dendrites	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LYMPH NODE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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SUMMARY OF TISSUE DISTRIBUTION AND REACTIVITY OF MONOCLONAL ANTIBODIES AGAINST COLON CANCER BY IMMUNOPATHOLOGY IN NORMAL FETAL, NORMAL ADULT AND CANCER TISSUE IN HUMAN SPECIMENS

SK 340  
4/12/85

TABLE IIA

MONOCLONAL ANTIBODIES

	85	28	6	29/36	29/15	479	15	174	86	70	152	19.9	307	218	29/26	68
<b>NORMAL ADULT TISSUES</b>																
BLOOD VESSEL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Endothelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Smooth Muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CAPILLARIES	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SKELETAL MUSCLE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SOFT TISSUE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SECRETIONS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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SUBRARY OF TISSUE DISTRIBUTION AND REACTIVITY OF MONOCLONAL ANTIBODIES AGAINST COLON CANCER BY IMMUNOPATHOLOGY IN NORMAL FETAL, NORMAL ADULT AND CANCER TISSUE IN HUMAN SPECIMENS

TABLE IIA

SK 340

4/12/85

MONOCLONAL ANTIBODIES

CANCER TISSUE	85	26	6	29/36	29/15	479	15	174	86	70	152	307	218	29/26	68
COLON CANCER	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LUNG CANCER	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BREAST CANCER	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BLADDER CANCER	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TESTICULAR CANCER	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MELANOMA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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SUMMARY OF TISSUE DISTRIBUTION AND REACTIVITY OF MONOCLONAL ANTIBODIES AGAINST COLON CANCER BY IMMUNOPATHOLOGY IN NORMAL FETAL, NORMAL ADULT AND CANCER TISSUE IN HUMAN SPECIMENS

SK 340  
4/12/85

TABLE IIA

MONOCLONAL ANTIBODIES

	85	28	6	29/36	29/15	479	15	174	86	70	152	307	218	29/26	68
41 CANCER TISSUE															
6 ASTROCYTOMA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10 LYMPHOMA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
KIDNEY CANCER	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0



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TABLE III REACTIVITY OF MOUSE MONOCLONAL ANTIBODIES GENERATED AGAINST COLORECTAL CANCERS. SEROLOGICAL TEST WITH CULTURE HUMAN CELLS

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CELLS	V-315	A-33	K-314	A-37	H-15	V-715	H-70	H-26
Immunoglobulin subclass	Y-1	Y-2b	Y-1	Y-1	Y-1	Y-1	Y-1	Y-2a
Molecular weight	gp 140		gp 170			gp 120	gp 31	gp 29
EPITHELIUM ORIGIN TUMORS								
COLON								
HT-29, SW-480, SW-403	+	+	+	+	+	+	+	+
SW-48, CACO-2, SW-1116	+	+	+	+	+	+	+	+
SK-CO-10, SK-CO-13	+	+	+	+	+	+	+	+
SW-1417, SW-1232, SK-CO-15	+	+	+	+	+	+	+	+
SW-620, SW-813, SK-CO-11	+	+	+	+	+	+	+	+
SW-1063, SK-CO-12, SK-CO-1	+	+	+	+	+	+	+	+
PANCREAS								
ASP-1, CAPAN-1, CAPAN-2	+	+	+	+	+	+	+	+
HEPATIC AND BILIARY								
SK-HEP-1, SK-CHL-2	+	+	+	+	+	+	+	+
LUNG								
J-82, CALU-1, CALU-5	+	+	+	+	+	+	+	+
CALU-6, SK-HES-1, SK-LU-1	+	+	+	+	+	+	+	+
SK-LC-1, -2, -4	+	+	+	+	+	+	+	+
SK-LC-5, -6, -8	+	+	+	+	+	+	+	+
SK-LC-9, -10, -11	+	+	+	+	+	+	+	+
SK-LC-15, -16, -17	+	+	+	+	+	+	+	+
SK-LC-18, -19, -23	+	+	+	+	+	+	+	+
SK-LC-24, -25, -28	+	+	+	+	+	+	+	+
SK-LC-LT	+	+	+	+	+	+	+	+
BLADDER								
253-J, SW-780, TTCBUP	+	+	+	+	+	+	+	+
5637, VM-CUB-1, VM-CUB-2	+	+	+	+	+	+	+	+
VM-CUB-3, 575-A, RT-4	+	+	+	+	+	+	+	+
63B-V	+	+	+	+	+	+	+	+
ECTODERM ORIGIN TUMORS								
BREAST								
MDA-MB-361, MCF-7, CAMA	+	+	+	+	+	+	+	+
SK-BR-3, MDA-MB-157, ATAB	+	+	+	+	+	+	+	+

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REACTIVITY OF ANTIBODIES GENERATED AGAINST GASTROINTESTINAL  
CANCERS: IMMUNOFLOUORESCENCE TESTS WITH FROZEN SECTIONS OF NORMAL  
HUMAN FETAL (F)\* AND ADULT (A) TISSUES

	V-215		A-33		K-314		A-37		H-15		V-715		H-70		H-2	
Normal Human Tissues	F	A	F	A	F	A	F	A	F	A	F	A	F	A	F	A
Colon	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Small intestine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stomach	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Esophagus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pancreas-Endocrine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Exocrine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Liver-Hepatocytes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Biliary epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lung-Bronchial epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Pneumocytes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Endothelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prostate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kidney-Glomerulus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Proximal Tubules	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Henle's Loop	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Distal Tubules	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Collecting Tubules	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Testis-Germ Cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Endocrine cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ovary	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Placenta-Syncytiotrophoblast	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Cytotrophoblast	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Uterus-Endometrium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Myometrium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cervix-Endocervix	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Exocervix	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Breast-Duct cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Acinar cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Adrenal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Skin-Epidermis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Adnexa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Melanocytes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brain-Neurons	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Glial cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Dendrites	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thyroid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spleen-White Pulp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Red Pulp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lymph Nodes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thymus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heart	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Endothelial cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fibroblasts	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cartilage	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Interstitial Matrix	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Secretions	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Reactivity of monoclonal antibodies with tissue sections is symbolized as follows:  
0, no immunofluorescence; ●, immunofluorescence, ○, heterogenous pattern of immunofluorescence.

\*Fetal tissues were obtained from a 14 weeks old fetus.

4/12/85

TABLE V REACTIVITY OF MONOCLONAL ANTIBODIES WITH THE TUMOR AND NORMAL ADJACENT FROZEN TISSUE SECTIONS OF CANCER PATIENT

Location	Patient	Monoclonal antibodies					
		V-215	A-33	K-31J	H-15	V-715	H-26
Rectal colon	AR	-	+	-	-	-	+
	AY	-	+	⊙	+	-	+
Sigmoid colon	BR	-	+	+	⊙	-	+
	BT	-	+	+	+	-	+
	CB	⊙	+	+	⊙	⊙	+
	CR	-	+	+	+	-	+
	DE	-	+	⊙	-	-	+
	DN	⊙	+	⊙	⊙	⊙	+
	FA	-	+	⊙	-	⊙	+
	PU	-	+	⊙	-	-	+
	GA	-	+	⊙	-	+	+
	GP	-	+	+	+	-	+
	GY	⊙	+	⊙	+	-	+
	BT	⊙	+	⊙	⊙	-	+
	MR	-	+	+	+	-	+
	MS	-	+	⊙	+	-	+
	NC	-	+	+	-	-	+
	ND	-	-	-	-	-	+
	OT	-	+	+	⊙	⊙	+
	PP	-	-	+	-	-	+
	RD	-	+	⊙	+	-	+
	RT	-	+	⊙	+	-	+
	RV	-	+	+	-	-	+
	VC	-	+	+	-	-	+
Left colon	BW	-	+	+	⊙	-	+
	HL	⊙	+	⊙	+	-	+
	MT	-	+	+	-	⊙	+
	SM	-	-	+	-	-	+
	ST	-	+	+	-	-	+
Right colon	BA	⊙	+	+	-	-	+
	BG	-	+	+	-	-	+
	PS	-	+	⊙	⊙	-	+
	GA	-	+	+	+	-	+
	BA	-	+	+	-	-	+
	HU	+	+	+	-	-	+
	KP	-	-	⊙	⊙	-	+
	SE	-	-	-	-	-	+

1 What is Claimed:

- 5 1. Monoclonal antibodies characterized by immunological binding to human gastro-intestinal cell antigens and wherein said monoclonal antibody is selected from the group consisting of V-215, K-314, V-715, AS-33, and AS-37.
- 10 2. Monoclonal-antibody-producing-hybridoma cell line formed by fusing a myeloma cell line and spleen cells derived from a mammal immunized with established culture cell lines of human gastrointestinal cell carcinomas, pancreatic tumor cell lines, wherein the monoclonal antibody is selected from the group
- 15 consisting of monoclonal antibody V-215, K-314, V-715, AS-33 and AS-37.
- 20 3. Panel of monoclonal antibodies for the diagnosis of human gastrointestinal cancer wherein the panel consists of two or more different monoclonal antibodies selected from the group consisting of V-215, K-314, V-715, AS-33, and AS-37.
- 25

- 1        4.    Panel of claim 3 wherein the human gastrointestinal  
         cancer diagnosed is human colon cancer.
- 5        5.    Panel of monoclonal antibodies for the diagnosis of  
         human gastrointestinal cancer wherein the panel  
         consists of two or more different monoclonal antibodies  
         selected from the group consisting of AS33, AS37,  
         V-214, V-715, CLH70, CLK314, CLT86, CLT307, HT 29-15,  
         and HT 29-26.
- 10       6.    Panel of claim 5 wherein the human gastrointestinal  
         cancer diagnosed is human colon cancer.
- 15       7.    Method for differentiating normal and abnormal  
         gastrointestinal cells which comprises contacting a  
         human gastrointestinal specimen containing  
         gastrointestinal cellular material with two or more of  
         the monoclonal antibodies of from the group consisting  
         of AS-33, AS-37, V-215, V-715, CLH70, CLK 314, CLT86,  
20       CLT307, HT29-15 and HT 29-26 and detecting the presence  
         or absence of immune complex formation with two or more  
         of said monoclonal antibodies indicating the presence  
         or absence of abnormality in the gastrointestinal  
         specimen.



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8. Method of Claim 7 wherein the abnormality is gastro-intestinal cancer.
- 5
9. Method of Claim 7 wherein the abnormality is colon cancer.
- 10
10. Method of Claim 7 wherein the specimen is contacted singly, serially or in combination with each of the panel monoclonal antibodies.
- 15
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